Manual



For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT -20 °C storage required immediately upon receipt



Manual

Bst DNA Polymerase, Exonuclease Minus 50,000 units/mL

1. Product description

Bst DNA Polymerase, Exonuclease Minus, 50,000 units/mL.

2. Product specifications

Storage buffer: 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton[®] X-100 (Rohm & Haas) and 50% Glycerol.

Stability: Bst DNA Polymerase, Exonuclease Minus is stable for one year from the date received if stored at -20 °C.

Recommended reaction conditions: 50 units Bst DNA Polymerase, Exonuclease Minus; 1X DNA Polymerase Buffer B containing 20 mM Tris-HCl pH 8.8, 10 mM (NH_4)₂SO₄, 10 mM KCl, 2 mM MgSO₄ and 0.1 % Triton X-100.

Activity determination: One unit catalyses the incorporation of 10 nmol of dNTP into acidinsoluble material in 30 minutes at 65 °C in 20 mM Tris-HCl pH 8.8, 10 mM (NH_4)₂SO₄, 10 mM KCl, 2 mM MgSO₄, 0.1 % Triton X-100, 30 nM M13mp18 ssDNA, 70 nM M13 sequencing primer(-47) 24 mer, 200 µM dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [³³P]dCTP) and 0.1 mg/ml BSA.

Absence of endonuclease or nicking activity: Incubation of 50 units of Bst DNA Polymerase, Exonuclease Minus with 1 µg of pUC19 DNA for 16 - 18 hours at 37 °C resulted in no smearing of bands detected by agarose gel electrophoresis.

Absence of exonuclease activity: Incubation of 50 units of Bst DNA Polymerase, Exonuclease Minus with 1 μ g of *Hin*dIII-cut lambda DNA for 16 hours at 37 °C and 65 °C resulted in no smearing of bands on agarose gels. Single stranded and double stranded exonuclease activities were tested by incubating 10 μ L of enzyme at 50 units/ μ L with radiolabeled DNA substrate for one hour at 37 °C and 65 °C, resulting in less than 0.1% release of TCA-soluble counts.

Purity: >90% pure by SDS PAGE. No detectable DNA contamination. 10 μ L of enzyme at 50 units/ μ L of the sample was tested for *E. coli* genomic DNA contamination by PCR amplifying with the *E. coli* 16S ribosomal primers.

Heat inactivation: 80 °C for 20 minutes.

3. Product designations and kit components

Product	Concentration	Kit size	Catalogue number	Reagent description	Part number	Volume
Bst DNA Polymerase, Exonuclease Minus 50,000 units/ml	50,000 units/mL	10,000 units	30028-1	Bst DNA Polymerase, Exonuclease Minus (50 units/µL)	F93636-1	200 µL
				10X DNA Polymerase Buffer B	F98637-1	4.8 mL

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Bst DNA Polymerase, Exonuclease Minus 50,000 units/mL

4. Storage conditions

Store all kits and components at -20 °C



5. Applications

- 1. DNA sequencing through high GC regions^{1, 2}
- 2. Rapid Sequencing from nanogram amounts of DNA template³

6. References

- 1. Griffin H and Griffin A 1994 PCR Technology, 228-229.
- 2. McClary J et al. 1991 J. DNA Sequencing and Mapping, 1, 173-180
- 3. Mead DA et al. 1991 Biotechniques, 11, 76-87.

7. Technical support

If you require any further support, please do not hesitate to contact our Technical Support Team: <u>techsupport@lgcgroup.com</u>.

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